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Does the Early Social Environment Affect Structure and Consistency of Personality in Wild-Type Male Rats?

ABSTRACT: Animal personality has been extensively studied from a functional and evolutionary point of view. Less attention has been paid to the development of personality, its phenotypic plasticity, and the influence of manipulation of early environmental factors. Here we describe the effects of manipulating the sex ratio of the litter, at postnatal day (pnd) 3, in wild-type rats, on personality traits in adulthood. We measured the treatment effects on aggression, defensive burying, and open field behavior at pnd 90 and 120, as well as on their contextual generality, and stability over time (differential and structural consistency). Main effects of litter composition were found on open field behavior at pnd 120 but not on the other behaviors. Since correlations between behaviors changed over time irrespective of the specific treatment, whereas in previous studies on unmanipulated litters this was not the case we suggest that early handling may disrupt adult personality traits. Overall the data indicate that personality is less stable over time than often assumed, having both proximate and ultimate implications. © 2011 Wiley Periodicals, Inc. *Dev Psychobiol* 53: 614–623, 2011.

Keywords: coping style; behavioral syndrome; temperament; litter composition; ontogeny; early life environment; structural consistency; social behavior; explorative behavior; *Rattus norvegicus*

INTRODUCTION

Over the past decade, the existence of consistent individual differences in a wide number of vertebrate and invertebrate species has been convincingly demonstrated (see Golsing & John, 1999). Although this phenomenon has been termed in different ways such as “coping style,” “temperament,” “behavioral profile, behavioral syndrome,” and personality (Stamps & Groothuis, 2009) it will be, following current mainstream literature, addressed as “personality” in this article. Animal personality is defined as individual

differences in behavior that are consistent across time and contexts (Stamps & Groothuis, 2009).

Apart from the many descriptive studies, animal personality has mainly been studied from a functional and evolutionary perspective (Biro & Stamps, 2008; Dall, 2004; Dingemanse & Reale, 2005; McNamara, Stephens, Dall, & Houston, 2009; Nettle, 2006; Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson, & Ziemba, 2004; Stamps, 2007; Wolf, van Doorn, Leimar, & Weissing, 2007; Wolf, van Doorn, & Weissing, 2008). In addition, several studies have investigated its underlying physiology, partly in relation to potential implications for research and treatment of impaired health and disease, as different personalities may differ in their vulnerability to stress, depression/anxiety disorders, and sensitivity to pharmacological treatments (Cavigelli, 2005; Koolhaas, 2008; Koolhaas, de Boer, Coppens, & Buwalda, 2010; Meerlo, Horvath, Nagy, Bohus, & Koolhaas, 1999).

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There is a wealth of studies showing effects of early stress on single behavioral and physiological traits in adulthood in both the primate and rodent literature (for a review see Maestripieri & Mateo, 2009). However, very few studies have investigated developmental aspects of personality in terms of correlated behaviors. Knowledge of the ontogeny of personality is relevant for understanding the mechanisms, the function, and the evolution of animal personality (Stamps & Groothuis, 2010). Some studies focused on the genetic basis and heritability of personality traits (Reale, Reader, Sol, McDougall, & Dingemanse, 2007; Van Oers, de Jong, van Noordwijk, Kempenaers, & Drent, 2005) but how the environment interacts with genetic information in affecting personality development, how personality develops in the course of ontogeny, and to what extent personality shows (adaptive or maladaptive) phenotypic plasticity has hardly been addressed. A few studies have demonstrated that some environmental factors at (sub) adulthood can induce correlations between two traits or alter the rank order consistency of traits.

For example, in sub-adult sticklebacks, predation risk can induce a correlation between two traits (in this case boldness and aggressiveness) (Bell & Sih, 2007). In coral reef fish an increase in temperature affects boldness and alters personality structure (Biro, Beckmann, & Stamps, 2009). In adult trout, stress can affect personality structure (Ruiz-Gomez et al., 2008). Nevertheless, it is still an open question whether, to what extent and how persistent certain experiences early in life affect correlations among personality traits later in life. Only one study tested the effect of the early environment, in this case food deprivation in the chick phase, on later personality in two selection lines of great tits, finding interesting gene by environment interactions (Carere, Drent, Groothuis, & Koolhaas, 2005).

In altricial rodents, it is well known that the early postnatal social environment plays a key role in the organization of adult individual differences in behavior (see Laviola & Terranova, 1998 for a review). The early postnatal social environment includes the interaction between parents and offspring (quality/quantity of maternal care) and the interactions between siblings (sex and number of littermates). Different studies reported that both litter size and gender composition of the litter (Benus & Henkelmann, 1998; Mendl & Paul, 1990; Namikas & Wehmer, 1978) have long lasting effects on offspring behavior and physiology (Benus & Henkelmann, 1998; Brain & Griffin, 1970; Dimitsantos, Escorihuela, Fuentes, Armario, & Nadal, 2007; Mendl & Paul, 1990; Spencer & Tilbrook, 2009). In particular, the effects of litter gender composition on development of aggressive behavior in mice have been repeatedly

reported (Benus & Henkelmann, 1998; Mendl & Paul, 1990). However, to the best of our knowledge only the effect on separate behavioral traits and not on personality aspects in terms of correlations over time and among different behaviors has been considered. Therefore, the present article is aimed at studying the effect of early rearing conditions (in terms of variation of the sex ratio in the litter) on structure and consistency of personality at adulthood in an unselected line of wild-type rats.

The availability of previous data on adult personality structure (De Boer, van der Vegt, & Koolhaas, 2003; Koolhaas, De Boer, & Buwalda, 1999; Koolhaas, De Boer, & Buwalda, 2006; Koolhaas, De Boer, Buwalda, & Reenen, 2007; Koolhaas et al., 2010) in the wild-type Groningen (WTG) rats makes this strain a good model to investigate the ontogeny of personality. In WTG male rats, offensive aggression is positively correlated with defensive burying behavior from at least 4 months of age. This strain shows large individual variation in aggression compared to other strains, as well as changes in aggression with age as displayed in the resident-intruder paradigm. In particular the mean attack latency decreases from the age of 3- to 4-month to become stable in adult hood (De Boer et al., 2003). The defensive shock-prod burying test has been extensively used to measure the variation in defensive reaction to an aversive stimulus; this test has been recognized to give relevant information for characterizing (pro)active and reactive or passive coping responses (De Boer & Koolhaas, 2003; Koolhaas, Korte, et al., 1999).

Here we describe the long-term effects of manipulation of early litter sex ratio on the development of adult personality traits and its consistency over time in the WTG male rats. In rodents, natural variation of the sex ratio in the litter occurs both in the laboratory and in the wild (see James, 1997 for a review). We focused on three main behaviors: offensive aggression, defensive burying, and open field behavior. We first asked the question whether the early social environment would affect aggression by comparing the adult males derived from male biased litters with males from female biased litters. According to previous data in mice we expect aggression to increase in males from the female biased litters. Since in the adult male aggression is strongly correlated with burying behavior in WTG male rats, we next asked the question whether burying behavior would be affected in the same direction as aggressive behavior by litter composition (increase of the level of aggression corresponding to an increase of the level of burying in the female biased litters). With the same line of reasoning, exploration in the open field behavior was studied as well. Since exploration of a novel

environment often correlates with aggression (Adriaenssens & Johnsson, 2011; Rodríguez-Prieto, Martín, & Fernández-Juricic, 2010) or boldness (Groothuis & Carere, 2005) we were interested in testing whether this behavior is also linked to aggression or burying in WTG male's rats. In this case our prediction is that also exploration would increase along aggression and or burying by litter composition (Dingemanse et al., 2009). Finally, we investigated the stability over time both in personality structure and in single behaviors.

METHODS

Animals Housing and Breeding Procedure

For breeding, a total of 22 adult male–female pairs of WTG rats (*Rattus norvegicus*) were used. Rats were housed on 12:12 dark–light cycle (light of at 14 hr) in a temperature-controlled room (temperature $21 \pm 2^\circ\text{C}$; relative humidity 60%). Breeding pairs were housed in standard polycarbonate cages (Macro type III) for a week. Pregnant females were housed alone in standard (Macro type IV) cages until delivery of the litters. To establish the day of birth each cage was checked every 12 hr for the presence of litters and this day was considered postnatal day (pnd) 0. The cages contained sawdust as bedding, nesting material, and wooden sticks as enrichment. Food (Standard Lab Chow, Hope Farms, NL) and water was provided ad libitum. This research was performed with the approval of the Institutional Animal Care and Use Committee of the University of Groningen (DEC 5640A).

Experimental Design

On pnd 3 the litters were adjusted to the exact litter gender composition we chose for our design. The original litter size varied from 7 to 14 and out of 22 only 19 litters which contained enough males/females ratio to be adjusted to one of the two gender composition were used. In each litter the sex of pups was determined by external genital inspection and each litter was designated to be culled to a males biased (MB) rearing condition, containing five males and one female or to a females biased (FM) rearing condition, containing five females and one male. After having modified the gender composition, litters were kept undisturbed until weaning at pnd 28. At weaning the mothers were removed from the cages and the pups were marked with none to five rings on the tail with a permanent marker to allow the individual recognition of each pup. On pnd 45, right before sexual maturity (ranging from pnd 45 to 65), the experimental rats were selected as follows: for the MB litters one male out of each litter was randomly selected as an experimental animal. For FB litters the one male was always assigned as experimental. The rest of the litter was not part of the experiment and some of the female were sterilized through a tubal ligation and these were randomly assigned as standard companions for the experimental males. On pnd 55 experimental

males were re-housed in pairs with an unknown sterilized (non-littermate) female in standard cages until the end of the experiment. Overall we obtained two experimental groups: MM males ($n = 10$) from the male biased nests and FM males ($n = 9$) from the female biased nests.

Behavioral Tests

Adult male rats were tested in three different behavioral tests: the open field, the defensive burying and the standard resident–intruder test. Since one of our aims was to measure the same behaviors of the same individuals at different ages to check for consistency over time FM and MM males were tested twice in the same battery of tests. The first series of tests was performed at early adulthood on pnd 90, and the second one on pnd 120 (adulthood). All tests were repeated in the same order.

Open Field Test. A round open field, of 120 cm diameter, made of gray plastic with a white Plexiglas floor was used as a test arena (Schmitt & Hiemke, 1998; Steiniger & Kretschmer, 2004). The floor was virtually divided in three areas: a center area of 40 cm diameter, a middle area of 20 cm radius, and a peripheral area of 20 cm radius. The test was carried out during the dark phase between 15.00 and 18.00 hr. Subjects were placed in the center area and movements in the arena were tracked by Ethovision (Noldus Information Technology, Wageningen, The Netherlands) systems for 10 min. The arena was cleaned after each test trial. The following parameters were included in the analysis: total distance moved in the whole arena as a measure of exploration (Russell, 1983) defined as “exploratory activity;” time spent in the peripheral zone and latency to reach the peripheral zone were used as a measure of anxiety-like behavior.

Shock-Prod Burying Test. The defensive burying test was conducted in a $24 \times 24 \times 36$ Plexiglas chamber with a hole of approximately 1 cm diameter at one side near the bottom of the cage. After a habituation period of at least 2 days the animals were tested 1–3 hr after the dark-phase had started. The electric prod was inserted through the cage's hole and was electrified until the rat touched the prod and received a mild electric shock. After contact with the rat, the electrical current was switched off for the rest of the test. The behavior of the rat was recorded for 10 min after having inserted the prod. The percentage time spent burying defined as “burying behavior” and the latency to start burying after the shock defined as “burying latency score” was included in the analysis (De Boer & Koolhaas, 2003).

Resident–Intruder Test. The standard resident–intruder test has been extensively used to assess levels of offensive aggression in rodents (De Boer et al., 2003; Koolhaas, Schuurman, & Wiepkema, 1980; Olivier, Mos, van Oorschot, & Hen, 1995). For the present test individual experimental males were housed in special cages measuring $80 \times 55 \times 50$ with front clear plastic walls that allows observation of aggression. Each male was housed together with his own female for a period of 5 days before the test to avoid social isolation and

promote territorial behavior. After the habituation period the level of offensive aggression was tested in the home cage of the resident (experimental MM or FM rats) during a confrontation with an intruder consisting of an unfamiliar co-specific Wistar rat of same age and sex. In total 19 intruders for each aggression test were used, each intruder is shuffled between the different resident during the 4th day of test in such a way that the resident received each day an unknown intruder. The standard resident–intruder consisted of 4 consecutive days of testing: during the first 3 days only the latency time to the first attack of the resident defined as “attack latency score” was scored with the intruder male being removed from the cage after the attack. On the 4th day the attack latency score together with full range of behavioral elements expressed by the resident towards the intruder was recorded for 10 min. Companion females were removed from the home cages half an hour before the test started and housed individually until the end of the test; all the tests were performed during the dark phase between 15.00 and 18.00 hr. The intruders were marked and randomly assigned each of the 4 days to a different resident.

In the present study the following categories were scored through The Observer 5.0 (Noldus) summed as “offensive aggression” and expressed as a total percentage on time: lateral threat, clinching, and keep down, chasing, upright posture. The offensive aggression and the attack latency score measured on the 4th day of test were used for statistical analyses.

Statistical Analysis

Statistical analyses were performed using SPSS 16 PC package, two-tailed p -values of $\leq .05$ were considered statistically significant. Normality of data was accepted or rejected according to the Shapiro–Wilk test. Not normally distributed variables were transformed (log or square root) in order to achieve normality, where normality was not achieved, variables were analyzed via non-parametric statistics. For each of the parameters measured we tested for differences between the two litter gender compositions using a two-tailed independent t -test or a Mann–Whitney U -test. Preliminary analysis showed the presence of different linkages (contextual generality) between the following three parameters: offensive aggression, burying behavior, and exploratory activity. Therefore, we selected those to investigate the structural consistency of personality (stability of linkage between two behaviors at different ages Stamps & Groothuis, 2010). To test the effect of treatment on the relation between combinations of two different behaviors we used a GLM with one of the behaviors as dependent variable, the other as covariate and treatment as fixed factor, allowing the interaction between the last two. Further analyses were conducted by using Pearson correlations. To test the extent of structural consistency of personality (whether linkages between two traits are consistent over time) we tested also whether correlations between different behaviors significantly changed over time (Steiger, 1980). The same approach was used to investigate the differential consistency (or broad sense repeatability, Stamps & Groothuis, 2010) of behaviors over time: This

provides information on whether individuals maintain their position in the frequency distribution of a particular behavior relative to other individual over time. The mean-level consistency was investigated using a one-way ANOVA for repeated measure with the rearing condition as factor. This analysis gives information about age-related changes in behavior over time at a mean group level (Roberts & DelVecchio, 2000).

RESULTS

Effect of Treatment on Single Behaviors at Postnatal Day 90

At early adulthood, almost no effect of rearing condition on the single behaviors was found, except for a non-significant trend in latency to bury the prod (see Tab. 1).

Effect of Treatment on Single Behaviors at Postnatal Day 120

At this age, rearing condition did have an effect on the open field behavior. MM spent significantly longer time in the peripheral area, showing also an almost significant trend to reach the peripheral area faster (see Tab. 2). The percentage of offensive aggression displayed by MM tended to be higher than in MF males. For the other parameters neither any significant differences nor trends were found (see Tab. 2).

Context Generality

Since no effects of the treatment or its interaction with the covariate on the dependent variables selected (see Statistical Analysis Section) was found ($p > .1$), the linkages between the three different behaviors investigated (offensive aggression, burying behavior, and exploratory activity) were analyzed regardless of the rearing condition by means of Pearson correlations. As expected, at early adulthood (pnd 90) a close to significance positive correlation between the percentage of offensive aggression and the percentage of defensive burying behavior was found [Pearson: $r = .452$, $p = .052$] (Fig. 1a). At later adulthood (pnd 120) this link between offensive aggression and defensive burying is not significant anymore [Pearson: $r = -.157$, $p = .533$] (Fig. 1b). At early adulthood, there is no significant correlation [Pearson: $r = .264$, $p = .275$] between defensive burying and open field behavior (Fig. 2a). This association seems to develop at later adulthood since at pnd 120 a significant positive correlation between the percentage of burying behavior and the distance moved in the open field arena [Pearson: $r = .483$, $p = .036$] was found (Fig. 2b). In male rats offensive aggression and open field behavior are not

Table 1. Effect of Treatment on Single Behaviors at pnd 90

Behavior	MM	MF	Statistic
Offensive aggression (% time, s)	$X = 26.74, SE = 6.28$	$X = 27.88, SE = 6.66$	$t(17) = -.124, p = .903$
Attack latency score (s)	mdn = 54.34, 25th perc. = 27.30, 75th perc. = 179.99	mdn = 69.44, 25th perc. = 45.48, 75th perc. = 390.04	$U = 32.00, p = .315$
Burying behavior (% time, s)	$X = 27.16, SE = 7.21$	$X = 26.13, SE = 5.60$	$t(17) = .111, p = .913$
Burying latency score (s)	mdn = 45.30, 25th perc. = 28.86, 75th perc. = 82.94	mdn = 54.40, 25th perc. = 52.46, 75th perc. = 122.68	$U = 24.00, p = .095$
Exploratory activity (cm)	$X = 4956.87, SE = 167.29$	$X = 5073.69, SE = 202.01$	$t(17) = -.449, p = .659$
Time spent in the periphery (s)	$X = 492.62, SE = 7.57$	$X = 500.86, SE = 10.03$	$t(17) = -.664, p = .515$
Latency to reach the periphery (s)	mdn = 6.7, 25th perc. = 3.05, 75th perc. = 9.20	mdn = 5.4, 25th perc. = 1.01, 75th perc. = 20.20	$U = 43.00, p = .905$

MM = males from male biased litters; MF = males from female biased litters.

Data are represented as average (X) and standards error (SE) or median (mdn) followed by 25th and 75th percentiles; last column refers to statistical testing of the differences between the treatments: Mann-Whitney (U) or t -test (t) followed by p -value.

significantly correlated to each other, neither at pnd 90 [Pearson: $r = -.198, p = .417$] nor at pnd 120 [Pearson: $r = .145, p = .578$].

Differential and Structural Consistency

The Pearson correlations between early and late adulthood (pnd 90 and 120) were low and not significant for

all behaviors (Tab. 3), indicating a lack of differential consistency.

The mean-level consistency analysis revealed no age effect for burying behavior [$F = 1.088, p = .311$] after having removed the interaction with the treatment. A trend in the interaction between treatment and age was found in aggressive behavior [$F = 3.674, p = .073$]. After removing the interaction no main effect was

Table 2. Effect of Treatment on Single Behaviors at pnd 120

Behavior	MM	MF	Statistic
Offensive aggression (% time, s)	$X = 40.22, SE = 5.16$	$X = 26.26, SE = 6.47$	$t(16) = 1.848, p = 0.083$
Attack latency score (s)	mdn = 55.70, 25th perc. = 21.37, 75th perc. = 141.50	mdn = 94.96, 25th perc. = 20.52, 75th perc. = 359.96	$U = 28.00, p = .536$
Burying behaviour (% time, s)	$X = 26.52, SE = 6.58$	$X = 39.01, SE = 8.21$	$t(17) = -1.198, p = .247$
Burying latency score (s)	mdn = 65.10, 25th perc. = 29.19, 75th perc. = 212.03	mdn = 45.28, 25th perc. = 25.04, 75th perc. = 188.18	$U = 32.00; p = .315$
Exploratory activity (cm)	$X = 4030.66, SE = 357.19$	$X = 4813.38, SE = 303.43$	$t(17) = -1.650, p = .117$
Time spent in the periphery (s)	mdn = 553.30, 25th perc. = 529.35, 75th perc. = 586.7	mdn = 527, 25th perc. = 529.35, 75th perc. = 539	$U = 19.00; p = .035$
Latency to reach the periphery (s)	mdn = 1.40, 25th perc. = 1.20, 75th perc. = 2.80	mdn = 2.80, 25th perc. = 1.50, 75th perc. = 8.60	$U = 21.00; p = .053$

For further legends see legend in Table 1.

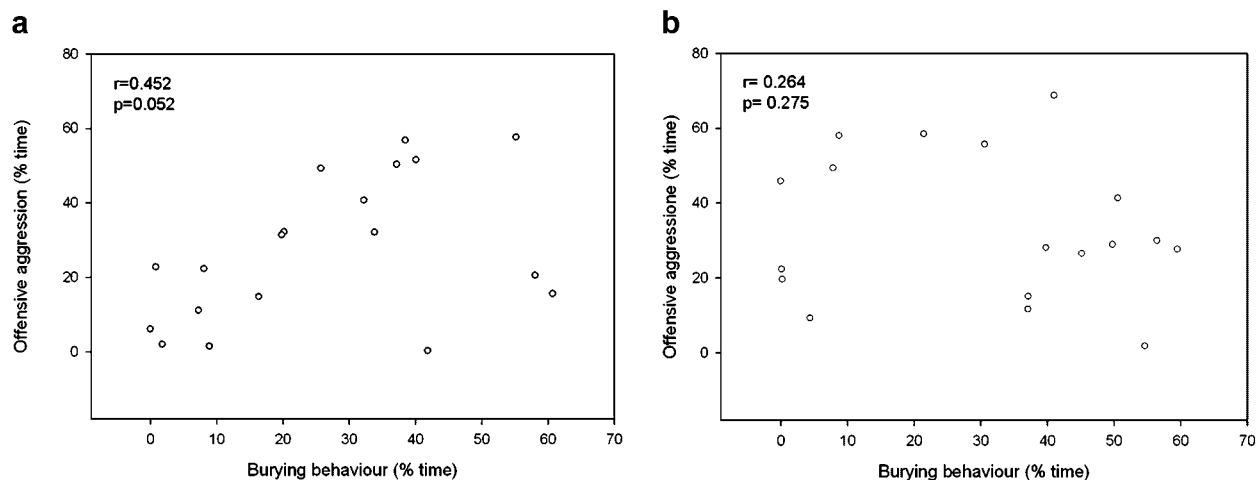


FIGURE 1 Relationship between individual scores of the percentage of offensive aggression measured in the resident intruder test and defensive burying measured in the shock-prod burying test, including results of Pearson correlation tests. (a) Postnatal day 90. (b) Postnatal day 120.

found for offensive aggression [$F = 1.022$, $p = .326$] suggesting that there is no increase or decrease of the average over time. For open field behavior a significant age effect [$F = 5.651$, $p = .029$] was found suggesting inconsistency of this trait over time: in particular the total distance moved in the arena significantly decreased from pnd 90 to 120.

Testing differences in personality *structure* over time (structural consistency) revealed a significant difference between the offensive aggression-burying correlation at pnd 90 and 120 [$Z = 2.33$, $p = .019$] suggesting that this linkage significantly changed over

time. In contrast, the burying-open field behavior correlation did not significantly differ between the two ages [$Z = -.69$, $p = .48$].

DISCUSSION

Our aims were twofold: using a rodent species for which personality has been established in adult animals, to investigate developmental stability in personality structure over time, which is first discussed, and testing the effect of litter composition on personality, which is discussed thereafter.

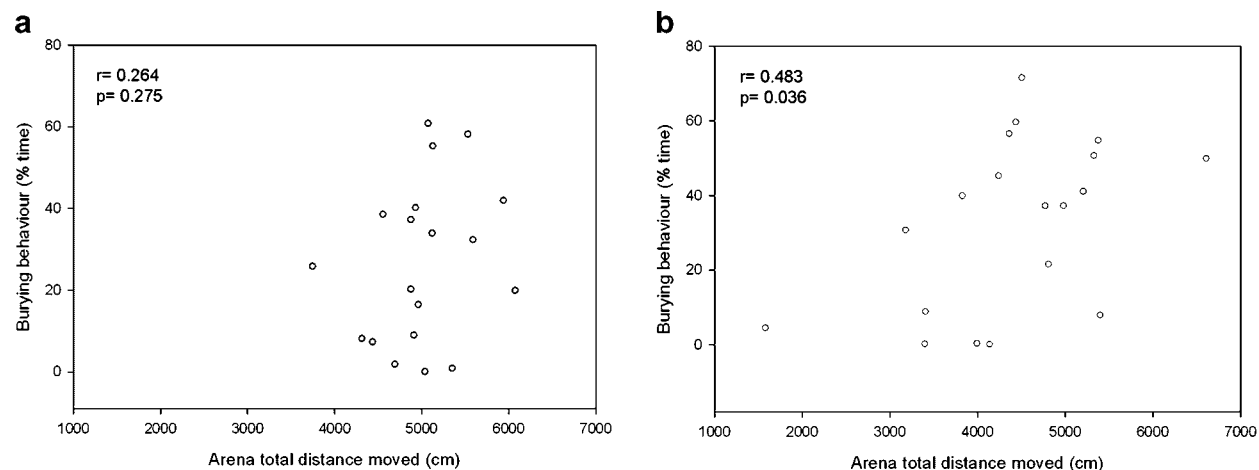


FIGURE 2 Relationship between defensive burying percentage of individual WTG male rats measured in the shock-prod burying test and the total distance moved in the arena measured in the open field test. (a) Postnatal day 90. (b) Postnatal day 120.

Table 3. Spearman Rank Order Correlations (*rs*, With *p*-Values Between Brackets) Between the Same Behavior at Two Different Ages (pnd 90 and 120)

Behavior	Pnd 90–120
Offensive aggression	.307 (.216)
Defensive burying	.333 (.163)
Exploratory activity	.167 (.495)

Personality Structure and Consistency at Adult Age

The positive relation between offensive aggression and defensive burying is well known in WTG male rats and has been replicated several times in animals at or older than 4 months of age. Our data suggest the presence of this personality structure also in younger animals, since we found such a relationship already in 3-month-old male rats irrespective of the rearing condition. However, this relationship between the two behaviors was no longer present in 4-month-old male rats. The statistical correlation comparison also indicated that the linkage significantly changed over time. This indicates that the two behaviors can be decoupled at a certain stage of development, presumably as a result of our manipulation during sensitive stages of development irrespectively of the specific sex ratio that was induced. This is because the relationship was repeatedly demonstrated in older animals that had not experienced any experimental treatment, in contrast to our animals. Hence, personality seems much less a limiting factor in phenotypic plasticity as previously assumed (e.g., Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson, & Ziemba, 2004).

Changes in linkage between two behavioral traits within individuals due to environmental manipulation have been demonstrated in several studies, for example, great tits (Carere, Drent, Privitera, Koolhaas, & Groothuis, 2005), sticklebacks (Bell & Sih, 2007), and trout (Ruiz-Gomez et al., 2008). Moreover, such changes have been observed during normal ontogeny as well (Bell & Stamps, 2004). We observed a marginally significant increase in aggression in MM at 4-month-old males. Perhaps the increased level of offensive aggression displayed by MM resulted in the disruption of the linkage between burying and aggression.

The opposite pattern was found in the relation between exploration activity in the open field and defensive burying. Here, the context generality appeared at late adulthood in 4-month-old rats whereas it was not present at 3-month-old males. In this case, the correlation comparison showed non-significant changes of such structure over time suggesting that this linkage was already present, although weaker, at earlier adulthood.

A lack of differential consistency over time for the same context was found for all behaviors measured, indicating that individuals do not perform consistently over time, despite the fact that both offensive aggression and defensive burying did not change in their mean-level over time.

In the human literature on personality it is generally accepted that stability in personality increases with age. For example, Roberts and DelVecchio (2000) found that rank-order consistency dramatically increases from childhood to the age of 30 to reach a plateau at the age of 50 (see also Caspi, Roberts, & Shiner, 2005). Similarly, rank-order stability in personality traits has

Table 4. Summary of Previous Finding Related to Early Gender Manipulation of the Litter and the Effect of it on Adult and Sub-Adult Aggression

Refs.	Litter Composition	Age	Aggression Measurements	Results
Benus and Henkelmann (1998)	1 male + 5 females (MF); 6 males (MM)	Sub-adult age	Aggressive interactions	MM higher frequency of aggressive interaction than MF
		Adulthood	Resident–intruder (attack latency score)	MF shorter latencies than MM
			Resident–intruder (attack latency score)	No significant differences in attack latency scores
Namikas and Wehmer (1978)	1 male + 5 females (MF); 6 males (MM)	pnd 60–65	Aggression test (aggression score)	MF higher aggression score than MM
Mendl and Paul (1990)	1 male + 3 females (MF); 4 males (MM)	pnd 55	Resident–intruder (attack latency score)	MF shorter latencies than MM
		pnd 90	Resident–intruder (attack latency score)	MF shorter latencies than MM

been shown to peak between 30 and 50 age (Ardelt, 2000). This suggests an age-related increase of the consistency of personality traits and that at young age major changes are more likely than later in life. Similarly, in animal personality literature a study on the development of shy/bold behavior in squid showed an increase of rank order consistency with age (Sinn, Gosling, & Moltschaniwskyj, 2008). A different situation was found in sticklebacks where in one of the two populations studied, stable correlations between traits across ontogeny were found but no stable rank-order consistency over time in single traits (Bell & Stamps, 2004). It might be that in our animals rank order consistency in defensive burying and offensive aggression develop at a later age than we measured at, for instance after 4-month. Especially for aggression it is known that WTG males show a considerable increase in aggression from pnd 90 to 120, with a much more stable performance thereafter (S.F. de Boer, unpublished results). Clearly, a lack of differential consistency can occur while the single traits do not change in mean level consistency over time.

A significant effect has been detected for exploration in the open field with the mean-level decreasing from 3- to 4-month of age (respectively, 1st and 2nd open field test). This decreasing level of exploration is indeed likely to be an age effect. A reduced exploration activity with age measured in the hole board test has also been reported in a study on temperament development in Wistar male rats (Ray & Hansen, 2005). However, we cannot fully exclude the possibility that this might be due to habituation to the open field test since the arena might not be perceived as a new environment during the second test resulting in a decrease in the level of exploration. To what extent age or habituation affected the change in correlation between open field behavior and defensive burying remains therefore an open question. Perhaps too much fear or anxiety during the first confrontation with the open field has masked an already existing correlation at the earlier age.

Effects of the Litter Gender Manipulation on Personality Traits at Adult Age

Opposite to our expectation, based on previous literature, the gender composition of the litter did not have strong effects on aggressive behavior and the other behaviors measured. Our data suggest the presence of an interaction between age and litter composition but this was a non-significant trend and in the opposite direction than expected based on previous data (see Introduction Section, Mendl & Paul, 1990). Our males that grew up within male biased litters (MM) displayed

a higher level of offensive aggression compared to FM males at month 4, but not at 3 months of age although the effect did not reach statistical significance. Interestingly, like for aggressive behavior, also for the open field behavior the effects of the rearing condition are only observed at late adulthood and not at the younger age. At 4-month of age, males reared in a male biased litter seem to be more fearful in facing a new environment, since they spent longer time in the peripheral zone of the arena and showed shorter latencies to reach the peripheral zone compared with FM males. A study by Laviola and Loggi (1992) seems to be in agreement with this, although this study used a slightly different litter composition.

Previous studies on the effects of litter composition on aggression (Benus & Henkelmann, 1998; Mendl & Paul, 1990; Namikas & Wehmer, 1978) reported consistent results showing that males reared in female biased litters (MF) display higher levels of aggression compared to males reared in all-male litters (MM) (see Tab. 3). An exception to this finding is shown by Benus and Henkelmann (1998) who did not find significant long-term effects (although short-term were found) neither in the latency of attack nor in the levels of aggressive interaction. Although previous studies reported different findings compared to our data there are some differences between the literature and the present data that might explain the inconsistent results. In this regard, it is important to take into account the slightly different experimental design, the different age in which subjects have been tested and the different model species used. In particular, in all available studies MM are males reared in only-male litters, whereas we used male biased litters containing one female. The reason for this litter composition was that we think that an only male litter would rarely occur in nature, making potential findings less relevant. Nevertheless, this presence or absence of one female in a litter may account for the difference between our study and the earlier ones. The different species used might have contributed as well, although the two rodent species are very similar with regard to ontogeny, physiology, and behavior differences in behavior (Bonthuis et al., 2010). Also, animals were usually tested at a younger age (from 50 to 90 pnd) in comparison to our data and a possible interaction between age and development of aggression might have occurred that may explain the different trend of aggressive behavior in our data (Tab. 4).

FINAL CONCLUSIONS

Overall, the results suggest the presence of plasticity in personality structure at adult age, as trait consistency

was low and correlations among behaviors changed in the course of late development. The possibility that differential consistency and structural consistency develop later in adulthood in accordance with in the development of human personality requires further investigation. The sex ratio of the litter had a non-significant effect on aggression, but in the opposite direction from what expected. Interestingly, the data suggest that early handling, but not the specific treatment, may have decoupled the link between aggression and defensive burying, but this needs further experimental testing.

NOTES

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